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EFFECTS OF SULPHUR DEFICIENCY ON METABOLISM IN TOMATO[1]

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(WITH ONE FIGURE)

Introduction

In connection with studies of protein and carbohydrate metabolism (**26, 27, 28, 29**), and of ammonium and nitrate nutrition (**42**), investigations have been made of effects of deficiency of various essential elements (**29, 30, 31**). This paper records the results of a similar series of experiments on deficiency of sulphur. Most of the experiments were conducted with tomato, although apple, narcissus, and asparagus were also used. This work does not include a study nor a review of the significance of sulphur in soils. An extensive review of the literature on this subject is given by JOFFE (**18**), together with the report of his own investigations. Later work on soil fertilization with sulphur-containing compounds is reported by FRAPS (**15**), who likewise reviews much of the literature.

Chemical methods

PLANT FRACTIONS.—The stem tip or upper 25 mm. of stem tissue was in some experiments removed and analyzed separately. The remainder of the stem was divided equally according to length into the two fractions which are termed *lower stem* and *upper stem*. The *petiole* fraction includes all vein and petiole tissue to which the mesophyll of the blade is not directly attached; the smaller veins are included with the other tissues

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The Kjeldahl, mineral, and sulphur determinations were made in the laboratory of C. S. CATHCART, for whose cooperation the writers wish to express their appreciation.

of the *blades* fraction, and have been estimated (**29**) to constitute about 35 to 50 per cent. by volume of that fraction. The analytical sample termed *roots* consists principally of fibrous roots and does not include the small taproot or the region that is intermediate in structure between the root and the stem.

The analytical plant fraction of paper-white narcissus which will be referred to as *centers* consists of the unexpanded floral organs and true leaves. Any top growth which was present is included with the centers for analysis and will be termed *centers* and *tops*. The storage scales, together with the disk-like stem of the bulb, are designated *storage tissue*. The entire root system is included in the analytical fraction *roots*. Details concerning the technique employed in separating these bulb fractions are given in another report (**28**).

NITROGEN FRACTIONS.—Fresh tissue only was employed for nitrogenous fractionation. The manner of extraction with water according to the method of CHIBNALL (**5**) has already been described (**29**). The term *protein nitrogen* is used to designate a group or groups of the more complex nitrogenous constituents, and includes both unextracted nitrogen and extracted coagulable nitrogen. The fraction termed by some investigators *total water-soluble nitrogen* is synonymous with *total extracted nitrogen*. In this publication no report is given of this fraction, as such a determination is chiefly indicative of the degree of grinding of the plant tissue, and is of doubtful metabolic significance (**28**). *Soluble nitrogen* as used in this work refers only to that fraction of extracted nitrogen which has been freed of coagulable nitrogen after being boiled and dilute acetic acid added (**5**).

Ammonia was estimated by the method of VAN SLYKE (**43**) on aliquots of the soluble-nitrogen fraction.

Amide nitrogen was determined by the usual procedure on aliquots of soluble nitrogen. It should be mentioned that for the determination of amide nitrogen sulphuric acid has always been employed in these laboratories, because with hydrochloric acid consistent results have not been obtained. Both CHIBNALL (**6**) and VICKERY (**44**) have recently reported that in the presence of nitrates, hydrochloric acid may not be employed in the determination of amide nitrogen, but that sulphuric acid is satisfactory. The humin nitrogen formed was found to be negligible in amount and the filtrate was employed for the estimation of basic nitrogen by precipitation with phosphotungstic acid (**29**).

Alpha amino nitrogen was estimated by the VAN SLYKE method on aliquots of the phosphotungstic acid filtrate (**29**).

Nitrate nitrogen was determined according to the procedure of SESSIONS and SHIVE (**37**) on aliquots of ammonia-free soluble nitrogen.

Other nitrogen was calculated as the difference between soluble nitrogen and the sum of the soluble-nitrogen fractions.

SULPHUR FRACTIONS.—Preliminary trials showed only a slight loss of volatile sulphur from freshly minced stem and blades of tomato that were dried in a current of air at 80° C. The amount lost was too small for quantitative estimation by PETERSON'S method (**32**), whereas cabbage leaves subjected to the same treatment yielded a considerable quantity of volatile sulphur. Consequently the determination of total sulphur in tomato tissue was carried out according to Official Methods (**2**), with minced tissue that had been dried rapidly at 80° C. in a current of air and had then been ground as previously described (**28**). For estimation of sulphur fractions, fresh tissue only was employed, because trials showed that the method of drying just described caused a marked decrease in proteins and a corresponding increase in sulphate-free soluble sulphur. The percentage of sulphates was found to be the same in dried as in fresh tissue. However, only in case of narcissus (tables X and XI) was dried material used for sulphate determinations. Aliquots of the protein or coagulum-free extract obtained as described under *nitrogen fractions* were used and the Official (**2**) procedure followed for determination of *sulphates*[2] and total *soluble sulphur*. *Protein sulphur* was computed as the difference between total sulphur and soluble sulphur.

Aliquots of the phosphotungstic acid precipitate obtained from the coagulum-free extract were analyzed for cystine according to the method of PLIMMER (**33**), but the quantity of cystine was insufficient for macrochemical estimation even in large aliquots of tomato stem or blade tissue. The extract had previously been subjected only to the mild acid hydrolysis and sodium carbonate treatment of the amide determination. Analyses for cystine and cysteine were also made by the colorimetric method of SULLIVAN (**38, 39, 40**); pigments present in the tomato-plant tissue interfered with quantitative determinations, although the results clearly indicated that traces of cysteine and cystine were present in the protein-free extract of whole tomato stems.

An attempt was made to adapt the colorimetric nitro-prusside reaction to a quantitative estimation of total S-H sulphur present in the protein-free extract of tomato-plant tissue; but the slight color which occurred was fleeting, and plant pigments interfered.

Microchemical methods

Microchemical tests were made on sections of plant tissue as recom-

[2] Any sulphites present are included in the sulphate determination. Nitrates at concentrations usually found in tomato were added to nitrate-free tomato stem extract. Recovery of sulphate was not apparently influenced by the added nitrate.

mended by ECKERSON (**14**). Some of these tests for sulphur-containing compounds are given later.

SULPHATES.—One per cent. benzidine in 3 per cent. hydrochloric acid was employed in testing for sulphates. It was the only reaction that was found satisfactory or sufficiently sensitive for testing for sulphates in plant tissue. This test in the presence of sulphates gave glistening white scale-like crystals of benzidine sulphate. Other reactions (**4**) were found more or less satisfactory in testing for sulphates in drops of expressed juice.

SULPHITES.—Extensive trials of many different reagents resulted in only one satisfactory method for testing for sulphites in plant tissue. To thin sections of plant tissue was added a drop or two of 10 per cent. sodium tetrathionate, followed in a few minutes by a drop of 10 per cent. barium chloride. Any sulphites present even in extreme dilution reacted at once to form barium thiosulphate crystals, which look much like starch grains but have a higher refractive index. The crystals often appear in pairs like two mushrooms, with a heavy black border, which is caused by light refraction. This test for sulphites is based upon the fact that salts of polythionic acid react with sulphites to form thiosulphates (**34**), which in turn react with barium chloride to form characteristic barium thiosulphate crystals.

GLUTATHIONE, CYSTEINE.—The nitro-prusside reaction as employed by KOZLOWSKI (**19**) and WHITE (**48**) was used as a test for total S-H or reduced sulphur. It is not, however, specific for either glutathione (**38, 39, 40**) or cysteine, although it has frequently been used by investigators as an index of the glutathione content of plants. Many studies arbitrarily reported as on glutathione are more strictly speaking investigations of the S-H sulphur content of plants. The colorimetric method of SULLIVAN (**38, 39, 40**) was used in making qualitative tests for cysteine on sections of plant tissue, on expressed juice, and on aliquots of protein-free extract of plant tissue.

CYSTINE.—Microchemically cystine was easily identified by its characteristic crystal form, comparative insolubility in water, high refractive index, and chemical reactions.

Experimental methods

Tomato plants (*Lycopersicon esculentum* Mill.) of the variety Marglobe were grown in sifted loam soil in individual 4-inch pots. On May 12, 1931, at the time of commencing experimental treatments, there were available 2500 plants. On that date, 1200 plants were selected for uniformity; the roots of each plant were washed free of soil and the two lower leaves were removed. Two hundred plants were used for initial analysis and 1000

plants were transplanted for experimental treatments to washed quartz sand in new 10-inch clay pots, one or two plants to a pot. The pots were set in shallow enamelware pans. For the period of these experiments no difference in growth was observable between plants that were grown one and those grown two in a pot.

Immediately after being transplanted from soil to sand culture, all of the plants were subjected to nutrient treatments.[3] Some of them received the complete or plus-sulphur, others the minus-sulphur, and a third group the minus-nitrogen solution, as indicated in table I. Also from time to time some of the plants were shifted from one nutrient treatment to another. Each pot received two liters of solution daily, and twice a week each culture was thoroughly flushed with distilled water, after which fresh nutrient solution was applied.

Sufficient iron for subsequent growth was present in the initial plants or possibly as impurities in the salts employed. Likewise, perhaps for the same reason, it was not found necessary to apply boron or manganese; at least the complete-nutrient plants which were allowed to mature grew luxuriantly and produced a heavy crop of fruit. Further, no apparent effect was produced on the minus-sulphur or minus-nitrogen or complete-nutrient plants by the addition of boron or manganese at the rate of one quarter part per million of each.

The plants were grown in a greenhouse at New Brunswick, during the spring and early summer of 1931, under conditions of temperature and humidity suitable for the commercial production of tomatoes. The plants were grown under the seasonal light conditions of the greenhouse, with the exception of some that were subjected to a period of continuous darkness at a practically constant temperature of 20° C. Each plant was kept pruned to a single stem, and blossoms were hand-pollinated daily; but pollen from flowers of one lot of plants was not used for pollination of flowers of another group. Thirty or more plants of a series were harvested for analysis at 7 A. M. on the several dates indicated in the tables of analytical data.

Results

On May 12, at the time the respective nutrient treatments (table I) were started, the initial tomato plants were about 30 cm. in height, dark

[3] The rather low (29) concentration of nitrate in the nutrient solutions (table I) was purposely maintained in order that there might be no accumulation of nitrates in the tissues of plants which were active in amino acid synthesis. A relatively high percentage of nitrate in a given lot of plants should then be of considerable interpretive value. If tomato plants receive a high external nitrate supply they become practically saturated with nitrate (29), even though they are rapidly synthesizing organic nitrogenous compounds.

green and somewhat soft or succulent; cell walls of stem fibers were little thicker than walls of adjacent parenchymatous tissues; and xylem elements were still comparatively thin-walled. The plants were very low in sugars. The stems contained only 6.10 per cent. dry matter (table II) and were practically devoid of starch except in the endodermis; on the other hand, they were high in organic nitrogen, high in organic sulphur, and contained considerable sulphate and nitrate (tables II and III). Traces of sulphite were also present, particularly in the phloem region of the stem.

TABLE I

PARTIAL VOLUME MOLECULAR CONCENTRATIONS OF SALTS USED*

	SOLUTION				
	K_2SO_4	$Ca(NO_3)_2$	$Mg(H_4PO_4)_2$	$CaCl_2$	KCl
Plus-S	0.0045	0.0090	0.0045	.....	.....
Minus-S	.....	0.0090	0.0045	.....	0.0045
Minus-N	0.0045	.....	0.0045	0.0090	.....

* Each solution has a total osmotic concentration value of 1.25 atmospheres.

Attention is also called to the fact that these very actively vegetative plants were high in sulphur. Tests of this protein-free aqueous extract showed that the extract contained only a trace of S-H sulphur (nitro-prusside reaction) (**18, 48**). However, rather intense reactions for total S-H sulphur were obtained, but only in the cambium region and in other meristematic tissues, namely, stem and root tips. SULLIVAN'S (**38, 39, 40**) reaction apparently indicated a faint trace of cysteine, likewise limited exclusively to meristematic tissue and not sufficient in quantity to be detected in the extract from whole stems. In view of the fact that according to the nitro-prusside reaction there was apparently much more total reduced sulphur than could be accounted for by the presence of cysteine (SULLIVAN'S method), it would seem not improbable that glutathione was mainly responsible for the positive S-H reaction of active tissues of the plant.

Under the microscope there was observed an occasional naturally occurring crystal of cystine in the comparatively alkaline tissue of the phloem (**9**). The protein-free aqueous extract did not contain a measurable quantity of cystine, as none could be detected by the method of SULLIVAN or by the procedure of PLIMMER (**33**).

PLANTS WHICH RECEIVED COMPLETE NUTRIENT SOLUTION

During the period of these experiments (May 12–July 8), the plus-sulphur or complete-nutrient plants (fig. 1) were moderately vegetative.

TABLE II

CONTINUOUSLY PLUS-SULPHUR AND CONTINUOUSLY MINUS-SULPHUR TOMATO PLANTS

SULPHUR AND NITROGEN FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF DRY MATTER AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER

Fractions determined	Date and nutrient treatment						
	May 12	June 9		June 19		July 8	
	Initial plants	Plus-S	Minus-S	Plus-S	Minus-S	Plus-S	Minus-S
Total sulphate-free S	0.328	0.136	0.112	0.162	0.138	0.166	0.120
Protein S	0.247	0.136	0.091	0.099	0.022	0.166	0.009
Sulphate-free soluble S	0.081	Trace	0.021	0.063	0.116	Trace	0.111
Sulphate S	0.127	0.111	0.011	0.124	Trace	0.173	Trace
Total S	0.455	0.247	0.123	0.286	0.138	0.339	0.120
Total nitrate-free N	2.297	1.315	1.365	1.054	1.255	1.007	1.383
Protein N	1.133	0.915	0.980	0.735	0.709	0.770	0.691
Nitrate-free soluble N	1.164	0.400	0.385	0.319	0.546	0.237	0.692
Basic N	0.280	0.173	0.093	0.071	0.080	0.052	0.110
Amino N	0.561	0.147	0.140	0.137	0.200	0.074	0.276
Amide N	0.272	0.030	0.086	0.049	0.127	0.038	0.158
Ammonia N	0.047	0.020	0.062	0.040	0.100	0.031	0.101
Other N	0.004	0.030	0.004	0.022	0.039	0.042	0.047
Nitrate N	0.279	0.080	0.150	0.060	0.183	0.065	0.301
Total N	2.576	1.395	1.515	1.114	1.438	1.072	1.684
Dry matter	6.100	13.000	14.150	15.250	17.400	18.800	20.350

TABLE III

CONTINUOUSLY PLUS-SULPHUR AND CONTINUOUSLY MINUS-SULPHUR TOMATO PLANTS

SULPHUR AND NITROGEN FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

FRACTIONS DETERMINED	DATE AND NUTRIENT TREATMENT						
	MAY 12	JUNE 9		JUNE 19		JULY 8	
	INITIAL PLANTS	PLUS-S	MINUS-S	PLUS-S	MINUS-S	PLUS-S	MINUS-S
Total sulphate-free S	0.020	0.018	0.016	0.025	0.024	0.031	0.024
Protein S	0.015	0.018	0.013	0.015	0.004	0.031	0.002
Sulphate-free soluble S	0.005	Trace	0.003	0.010	0.020	Trace	0.022
Sulphate S	0.008	0.014	0.002	0.019	Trace	0.033	Trace
Total S	0.028	0.032	0.018	0.044	0.024	0.064	0.024
Total nitrate-free N	0.140	0.171	0.193	0.161	0.218	0.189	0.281
Protein N	0.069	0.119	0.139	0.112	0.123	0.144	0.140
Nitrate-free soluble N	0.071	0.052	0.054	0.049	0.095	0.045	0.141
Basic N	0.017	0.022	0.013	0.010	0.014	0.010	0.022
Amino N	0.034	0.019	0.020	0.021	0.035	0.014	0.056
Amide N	0.017	0.004	0.012	0.007	0.022	0.007	0.032
Ammonia N	0.003	0.003	0.009	0.006	0.017	0.006	0.021
Other N	0.001	0.004	0.002	0.005	0.007	0.008	0.010
Nitrate N	0.017	0.010	0.021	0.009	0.032	0.013	0.061
Total N	0.157	0.181	0.214	0.170	0.250	0.202	0.342

The initial plants contained practically no starch, and it was not until June 9 that there began to occur considerable starch storage. At about that time many fruits were set. Although the plants were not extremely active nor soft, they were nevertheless dark green and did not lose any of their lower leaves during the period of the experiments. The rate of growth, however, was much slower than that of less fruitful plants that

FIG. 1. Tomato plants, June 23, 1931: from left to right, plus-sulphur and minus-sulphur. Compare relative diameter of stems of the two plants. Note that sulphur-deficient plant is so stiff and woody that no stake is required to support it; that it is lighter in color; and that a single fruit has developed whereas fruits have not yet set on the plus-sulphur plant.

received a complete nutrient solution with three times the concentration of nitrates.

On July 8, stems of the fruitful, moderately vegetative plants that were supplied with the complete nutrient solution (table I) contained the

following percentage of carbohydrates on a dry weight basis: reducing sugars 4.20, sucrose 4.35, and starch and dextrin 12.17. The moderately low concentration of nitrogenous constituents is indicated in tables II and III.

The percentage of organic sulphur and sulphates was about half that found in the initial plants. Further, most of the sulphate-free sulphur was in the form of protein (tables II and III). Microchemical tests also indicated that there was much less S-H sulphur than in the initial plants. Strong, positive reactions for reduced sulphur, however, were obtained in the vicinity of stem and root tips and in the cambium region, especially of the upper half of the stem.

SULPHUR-DEFICIENT PLANTS

Symptoms of sulphur deficiency developed very gradually, but by June 9 the upper half of the stem of the minus-sulphur plants was very small in diameter and produced no axillary shoots. The total stem length, however, was equal to or greater[4] than that of the plants supplied with the complete nutrient solution (fig. 1). The lower leaves especially, and by July 8 even the upper leaves, were yellowish green with purple veins. The newly formed leaflets were of small area and were spaced far apart on the rachis. Stems were stiff and woody, and the few fruits formed were set and matured early (fig. 1). The minus-sulphur plants looked as if they had been gradually but not completely limited as to their nitrogen supply. This appearance was borne out by the root systems, which had the general character of those usually produced by low-nitrogen plants (**20, 26, 29**). The roots were somewhat extensive, but of small diameter because there was practically no cambium nor secondary thickening. Cells did not show any deformity nor injury, however, as in calcium deficiency (**31**).

Macrochemical analyses for essential mineral elements showed that sulphur deficiency did not materially affect the concentration of most of the mineral elements. The concentration of calcium was somewhat high, however, caused apparently by heavy deposits of calcium oxalate, especially in the phloem. The phloem of the minus-sulphur plants was about pH 5.6 as compared with 7.0 in the complete-nutrient series. Other mineral deficiencies have been shown (**10, 30**) to result in plant tissue of comparatively low pH and high concentration of calcium oxalate. There was no accumulation of any other mineral element; in fact, computed on a dry-

[4] The limit of ability of the sulphur-deficient plants to continue stem elongation was not determined. A few specimens were retained until August 1, and even at that time stems were still increasing in length, although the last-formed 30 cm. of stem was not more than 2 mm. in diameter.

weight basis, analyses of July 8 show that the percentage of potassium in the stems of the plus-sulphur plants was 2.98 as compared with 1.99 in the sulphur-deficient series. The lower figure, however, represents a concentration of potassium that is more than adequate for good growth of tomato (**30**). The lower concentration is probably associated with the fact that the minus-sulphur plants had little meristematic tissue. Active tissue is notably high in potassium (**30**).

When analyzed on July 8, the stems of the minus-sulphur plants contained the following percentage of carbohydrates computed on a dry weight basis: reducing sugars 6.32, sucrose 7.00, and starch and dextrin 14.79. The anatomical structure also corresponded closely to that of typical nitrogen-deficient high-carbohydrate plants (**20, 30**). The stems had no active cambium except in and near the stem tip; the walls of both internal and external fibers were extremely thick; and there was a very high proportion of thick-walled xylem and collenchyma tissue.

Nitrates were not low; in fact, when the last harvest was made (tables II and III) the stems of the sulphur-deficient lot contained nearly six times the concentration of nitrate found in the complete-nutrient plants. Total organic nitrogen also was consistently a little higher in the minus-sulphur group; this was caused mainly by accumulation of basic, amino, and amide nitrogen. Ammonia also was high (tables II and III). The percentage of total organic sulphur was not materially different in the two groups of plants, but in the plus-sulphur series nearly all of it was protein; whereas in the plants lacking an external sulphate supply there was little protein sulphur but much soluble sulphate-free sulphur. This non-protein water-soluble organic sulphur gave negative tests for cysteine, cystine, and glutathione. Its form was not determined. The extremely small stem tip of the sulphur-deficient plants appeared, however, to give a very faint S-H reaction (nitro-prusside test).

The concentration of sulphur in different parts of the plant is indicated in tables IV and V.

Sulphur-deficient plants shifted to plus-sulphur nutrient treatment

A few seconds after sulphur-deficient plants were supplied with the complete nutrient solution, sulphates were observed adsorbed on the outer pectic layers of the root hairs, and almost immediately could be detected within them. Five minutes later sulphates appeared in the fine roots, and after six hours large quantities of sulphate were found in the base of the stem and smaller quantities in the remainder of the stem and in the leaves.

The appearance of sulphites in different parts of the plants was almost simultaneous with that of sulphates, although the maximum concentration

TABLE IV

CONTINUALLY PLUS-SULPHUR AND CONTINUALLY MINUS-SULPHUR TOMATO PLANTS

SULPHUR FRACTIONS EXPRESSED AS PERCENTAGE OF DRY MATTER AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER

PLANTS HARVESTED FOR ANALYSIS JUNE 30, 1931

MATERIAL	TREATMENT						
	TOTAL SULPHATE-FREE S		SULPHATE S‡	TOTAL S		DRY MATTER	
	PLUS-S	MINUS-S	PLUS-S	PLUS-S	MINUS-S	PLUS-S	MINUS-S
Tip of stem	0.227	0.131	0.189	0.416	0.131	7.89	17.26
Upper stem	0.152	0.111	0.131	0.283	0.111	17.10	19.00
Lower stem	0.119	0.125	0.096	0.215	0.125	20.50	21.70
Fibrous roots*	0.318	0.238	0.463	0.781	0.238	9.02	10.20
Upper petioles	0.221	0.020	0.279	0.500	0.020	11.95	10.32
Lower petioles	0.177	0.103	0.485	0.662	0.103	11.80	11.20
Upper blades	0.387	0.152	0.747	1.134	0.152	17.00	16.80
Lower blades	0.261	0.214	1.871	2.132	0.214	15.50	17.75
Flesh of ripe fruit	0.149	0.106	0.063	0.212	0.106	6.92	5.94
Seed of ripe fruit†	0.228	0.141	0.021	0.249	0.141	.....	.....

* Computed on ash-free basis.

† Computed as percentage of air-dry weight of seeds.

‡ Minus-S plants did not contain sufficient sulphate to be detected by macrochemical analysis (2).

TABLE V

CONTINUALLY PLUS-SULPHUR AND CONTINUALLY MINUS-SULPHUR TOMATO PLANTS
SULPHUR FRACTIONS EXPRESSED AS PERCENTAGE OF GREEN MATTER
PLANTS HARVESTED FOR ANALYSIS JUNE 30, 1931

MATERIAL	TREATMENT				
	TOTAL SULPHATE-FREE S		SULPHATE S‡	TOTAL S	
	PLUS-S	MINUS-S	PLUS-S	PLUS-S	MINUS-S
Tip of stem	0.018	0.022	0.015	0.033	0.022
Upper stem	0.026	0.021	0.022	0.048	0.021
Lower stem	0.024	0.027	0.020	0.044	0.027
Fibrous roots*	0.029	0.024	0.041	0.070	0.024
Upper petioles	0.026	0.002	0.034	0.060	0.002
Lower petioles	0.021	0.012	0.057	0.078	0.012
Upper blades	0.066	0.026	0.066	0.193	0.026
Lower blades	0.040	0.038	0.290	0.330	0.038
Flesh of ripe fruit	0.010	0.006	0.005	0.015	0.006
Seed of ripe fruit†	0.228	0.141	0.021	0.249	0.141

* Computed on ash-free basis.

† Computed as percentage of air-dry weight of seeds.

‡ Minus-S plants did not contain sufficient sulphate to be detected by macrochemical analysis (2).

of sulphite was not reached until about 24 hours following the shift to plus-sulphur treatment.

Six hours after absorption of sulphate the roots gave faint but positive reactions for S-H sulphur; somewhat later much stronger S-H reactions occurred, particularly in the cambium and phloem regions and in all tissues of the tops that were not entirely mature. Some, but only a small part, of the reduced sulphur was cystein (SULLIVAN reaction); and therefore by inference (page 567) it may be suggested that most of the reduced sulphur was glutathione.

Seventy-two hours after the shift to plus-sulphur treatment (June 23, tables VI and VII) there was found a decrease in nitrate and an increase in organic nitrogen. Accompanying the decrease in nitrate, strong reactions for nitrite were obtained in the then more alkaline phloem region of roots and tops. Tables VI and VII also show that following the shift to complete nutrient there occurred a marked and very rapid increase in percentage of protein sulphur. Total organic sulphur showed no increase on a percentage basis. This is probably because the plants increased rapidly in volume immediately following the shift. On an absolute amount basis there was unquestionably an increase in total organic sulphur.

The response to an application of sulphates was extremely rapid. The plants had turned much darker green within twelve hours. Even the lower yellow leaves turned green during that time, and by the end of the second and third day there was a marked increase in diameter of newly formed stem and in area of newly developing leaves.

TABLE VI

MINUS-SULPHUR TOMATO PLANTS SHIFTED TO PLUS-SULPHUR TREATMENT
SULPHUR AND NITROGEN FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF DRY MATTER AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER

FRACTIONS DETERMINED	DATE AND NUTRIENT TREATMENT		
	JUNE 19	JUNE 23	JUNE 30
	MINUS-S	MINUS-S TO PLUS-S*	MINUS-S TO PLUS-S*
Total sulphate-free S	0.138	0.093	0.121
Protein S	0.022	0.052	0.097
Sulphate-free soluble S	0.116	0.041	0.024
Sulphate S	Trace	0.017	0.025
Total S	0.138	0.110	0.146
Total nitrate-free N	1.255	2.253	1.066
Protein N	0.709	1.667	0.617
Nitrate-free soluble N	0.546	0.586	0.449
Basic N	0.080	0.111	0.097
Amino N	0.200	0.273	0.200
Amide N	0.127	0.130	0.082
Ammonia N	0.100	0.082	0.033
Other N	0.039	−0.010	0.037
Nitrate N	0.183	0.077	Trace
Total N	1.438	2.330	1.066
Dry matter	17.400	16.900	15.520

* After June 19, some of the minus-S plants were subjected to plus-S treatment.

TREATMENT IN DARKNESS

The plants which received the nutrient solution lacking nitrogen (minus-nitrogen plus-sulphur) were typical yellow, stunted, woody plants, very low in nitrogen and high in carbohydrates. After twelve days of continuous darkness there was as usual (**26, 29**) a marked drop in carbohydrates, a decrease in protein and a corresponding increase in amino and amide nitrogen (tables VIII and IX). Total nitrogen decreased in percentage because the plants increased greatly in volume and green weight, although there was no external nitrogen supply. The stems showed an

increase in length of 15 to 20 cm., and many new leaves expanded during the interval of twelve days without light. The temperature was about 20° C. In the nitrogen-deficient plants during the darkness period, catabolism seems also to have been the predominant phase, not only of nitrogen but also of sulphur metabolism (tables VIII and IX).

It may further be noted that the minus-nitrogen plants did not accumulate large quantities of sulphate, even though before the shift to

TABLE VII

MINUS-SULPHUR TOMATO PLANTS SHIFTED TO PLUS-SULPHUR TREATMENT
SULPHUR AND NITROGEN FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

FRACTIONS DETERMINED	DATE AND NUTRIENT TREATMENT		
	JUNE 19	JUNE 23	JUNE 30
	MINUS-S	MINUS-S TO PLUS-S*	MINUS-S TO PLUS-S*
Total sulphate-free S	0.024	0.016	0.019
Protein S	0.004	0.009	0.015
Sulphate-free soluble S	0.020	0.007	0.004
Sulphate S	Trace	0.003	0.004
Total S	0.024	0.019	0.023
Total nitrate-free N	0.218	0.381	0.165
Protein N	0.123	0.282	0.096
Nitrate-free soluble N	0.095	0.099	0.069
Basic N	0.014	0.019	0.015
Amino N	0.035	0.046	0.031
Amide N	0.022	0.022	0.013
Ammonia N	0.017	0.014	0.005
Other N	0.007	–0.002	0.005
Nitrate N	0.032	0.013	Trace
Total N	0.250	0.394	0.165

* After June 19, part of the minus-S plants were subjected to plus-S treatment.

darkness the sulphate-free soluble sulphur fraction was high (tables VIII and IX) as compared with that of the complete-nutrient plants (tables II and III). This fraction appeared to contain a trace of S-H sulphur but no cystine. Following the period of darkness, newly developed tissue of the minus-nitrogen plants gave apparently more intense S-H reactions.

The treatment in darkness of sulphur-deficient plants resulted in etiolation of newly expanded leaves but did not appear to affect in slightest degree the rate of stem elongation nor any other noticeable phase of growth

as compared with other minus-sulphur plants which were subjected to the seasonal light conditions of the greenhouse during the same period. Carbohydrates decreased materially in the minus-sulphur plants during the 12-day period without light, and there was some indication of catabolism of nitrogenous materials (tables VIII and IX). The same tables of data, however, indicate no material change in percentage nor quality of sulphur fractions.

TABLE VIII

MINUS-NITROGEN AND MINUS-SULPHUR TOMATO PLANTS SHIFTED FROM DAYLIGHT TO CONTINUOUS DARKNESS

SULPHUR AND NITROGEN FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF DRY MATTER AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER

FRACTIONS DETERMINED	NUTRIENT TREATMENT AND DATE			
	MINUS-N (PLUS-S)		MINUS-S (PLUS-NO_3)	
	JUNE 6	JUNE 18	JUNE 19	JULY 2
	NORMAL LIGHT	PART OF JUNE 6 PLANTS AFTER 12 DAYS' DARKNESS	NORMAL LIGHT	PART OF JUNE 19 PLANTS AFTER 12 DAYS' DARKNESS
Total sulphate-free S	0.154	0.118	0.138	0.154
Protein S	0.117	0.068	0.022	0.035
Sulphate-free soluble S	0.037	0.050	0.116	0.119
Sulphate S	0.071	0.138	Trace	Trace
Total S	0.225	0.256	0.138	0.154
Total nitrate-free N	0.828	0.853	1.255	1.683
Protein N	0.657	0.531	0.709	0.820
Nitrate-free soluble N	0.171	0.322	0.546	0.863
Basic N	0.053	0.060	0.080	0.174
Amino N	0.082	0.164	0.200	0.395
Amide N	0.029	0.107	0.127	0.120
Ammonia N	None	Trace	0.100	0.107
Other N	0.007	−0.009	0.039	0.067
Nitrate N	None	None	0.183	0.257
Total N	0.828	0.853	1.438	1.940
Dry matter	15.550	12.740	17.400	12.530

Certain other experiments are considered in the discussion that follows. Results of cooperative studies with Dr. ECKERSON on sulphate and nitrate reduction are also included.

Discussion

EXTERNAL RESPONSES.—It has been found (**31**) that if seedling tomato plants are transplanted to sand cultures deficient respectively in nitrogen

(**20, 29**), phosphorus (**10**), or potassium (**30**), the effects of nitrogen or phosphorus deficiency are evident in a comparatively short time, but conspicuous symptoms of lack of potassium (**30**) are not usually apparent until much later. Although symptoms of lack of these respective elements may not occur simultaneously, the effects upon the general appearance of the plants are similar. The lower leaves and lower stem are yellowish green tinged with the purplish blue of anthocyanin pigments, and the uppermost

TABLE IX

MINUS-NITROGEN AND MINUS-SULPHUR TOMATO PLANTS SHIFTED FROM DAYLIGHT TO CONTINUOUS DARKNESS

SULPHUR AND NITROGEN FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

	NUTRIENT TREATMENT AND DATE			
	MINUS-N (PLUS-S)		MINUS-S (PLUS-NO_3)	
FRACTIONS DETERMINED	JUNE 6	JUNE 18	JUNE 19	JULY 2
	NORMAL LIGHT	PART OF JUNE 6 PLANTS AFTER 12 DAYS' DARKNESS	NORMAL LIGHT	PART OF JUNE 19 PLANTS AFTER 12 DAYS' DARKNESS
Total sulphate-free S	0.024	0.015	0.024	0.019
Protein S	0.018	0.009	0.004	0.004
Sulphate-free soluble S	0.006	0.006	0.020	0.015
Sulphate S	0.011	0.018	Trace	Trace
Total S	0.035	0.033	0.024	0.019
Total nitrate-free N	0.129	0.109	0.218	0.211
Protein N	0.102	0.068	0.123	0.103
Nitrate-free soluble N	0.027	0.041	0.095	0.108
Basic N	0.008	0.008	0.014	0.021
Amino N	0.013	0.021	0.035	0.049
Amide N	0.005	0.014	0.022	0.016
Ammonia N	None	Trace	0.017	0.013
Other N	0.001	−0.002	0.007	0.009
Nitrate N	None	None	0.032	0.032
Total N	0.129	0.109	0.250	0.243

leaves and tip of the stem are rather dark green and may remain so for a considerable period. On the other hand, calcium-deficient tomato plants (**31**) are distinctive in appearance, in that the upper half of the plant is yellow rather than green, and the lower half instead of being yellowish is rather dark green. The lower leaves often remain green even after death of stem tips and complete loss of chlorophyll from the upper half of the plant.

TABLE X

PAPER-WHITE NARCISSUS BULBS GROWN IN SAND CULTURE FROM NOVEMBER 16 TO DECEMBER 13*

SULPHUR AND NITROGEN FRACTIONS EXPRESSED AS PERCENTAGE OF DRY MATTER AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER

FRACTIONS DETERMINED	NUTRIENT TREATMENT AND PART OF PLANT					
	MINUS-N (PLUS-S)			COMPLETE (PLUS-NO_3)		
	CENTERS AND TOPS	STORAGE TISSUE	ROOTS	CENTERS AND TOPS	STORAGE TISSUE	ROOTS
Total sulphate-free S	0.150	0.200	0.280	0.255	0.220	0.220
Sulphate S	0.130	0.130	0.720	0.075	0.140	0.780
Total S	0.280	0.330	1.000	0.330	0.360	1.000
Total nitrate-free N	2.940	1.330	3.075	3.340	1.990	4.925
Nitrate N	None	None	None	None	Trace	0.845
Dry matter	11.300	30.080	6.030	10.000	27.270	6.470

* Results have been reported of analyses of these bulbs for carbohydrate and nitrogenous fractions (28).

TABLE XI

PAPER-WHITE NARCISSUS BULBS GROWN IN SAND CULTURE FROM NOVEMBER 16 TO DECEMBER 13

SULPHUR AND NITROGEN FRACTIONS EXPRESSED AS PERCENTAGE OF GREEN MATTER

FRACTIONS DETERMINED	NUTRIENT TREATMENT AND PART OF PLANT					
	MINUS-N (PLUS-S)			COMPLETE (PLUS-NO_3)		
	CENTERS AND TOPS	STORAGE TISSUE	ROOTS	CENTERS AND TOPS	STORAGE TISSUE	ROOTS
Total sulphate-free S	0.017	0.060	0.017	0.026	0.060	0.014
Sulphate S	0.015	0.039	0.043	0.007	0.038	0.050
Total S	0.032	0.099	0.060	0.033	0.098	0.065
Total nitrate-free N	0.332	0.400	0.185	0.334	0.543	0.319
Nitrate N	None	None	None	None	Trace	0.055

Sulphur-deficient tomato plants, however, are easily distinguished from those lacking calcium. In the sulphur-deficient plants of these experiments the stem tips of the former, although small in diameter, were not dead nor even injured, and the upper portion of the plant was darker green than the lower. In some respects these plants were similar to plants deficient re-

spectively in nitrogen, phosphorus, and potassium, in that the stems and petioles were stiff and woody, anthocyanin was conspicuously present, and the foliage was light yellowish green, particularly on the lower half of the plant. They exhibited an apparently distinctive and remarkable capacity for stem elongation (fig. 1). Many of the plants lacking sulphur had even longer stems and were taller than those which received the complete nutrient solution. Yet the stems were much more woody, and, as shown in figure 1, were of extremely small diameter.

Low-nitrogen plants (**9, 20, 29**) never exhibit such a growth response unless they are subjected to some such treatment as darkness, shading (**26, 29**), or heavy pruning (**20**); and apparently phosphorus- (**10**) or potassium-deficient tomato plants exhibit rapid stem elongation only following or accompanying carbohydrate depletion, which often occurs only shortly before death of the plant.

PROTEOLYSIS.—The breaking down of proteins to simple water-soluble organic nitrogenous compounds as amino acids and asparagine, and the reutilization of these and similar compounds in the development of new tissues, is a process in plants that commonly follows or accompanies increase in moisture content and decrease in percentage of dry matter and stored carbohydrates (**8, 26, 29**). In seed germination there is available a conspicuous example of this process. There are present conditions of abundant moisture, and in the seed there occur continual decrease in stored carbohydrates or fat and rapid breaking down and utilization of storage proteins.

Nitrogen-deficient tomato plants are high in carbohydrates and obviously low in total nitrogen (**9, 20, 29**). Further, much of the nitrogen consists of complex relatively immobile storage proteins (**29**). Breaking down of these proteins apparently does not occur except when preceded or accompanied by loss of carbohydrates, as when the plants are placed in continual darkness (**29**). There then occur rapid stem elongation, decrease in complex storage proteins, and increase in percentage of the simpler water-soluble forms of organic nitrogen.

Tomato plants lacking an external potassium (**30**) or phosphate (**10**) supply are, except during the last stages of growth, high-carbohydrate plants; and there is little proteolysis or vegetative extension unless the plants are shaded, placed in darkness, or otherwise treated to decrease the carbohydrate reserves. However, shortly before death of potassium- or phosphate-deficient plants, there occurs a decided decrease in carbohydrates, accompanying which there is, apparently, considerable breaking down and utilization of protein and fairly rapid stem elongation. Death of the plant within a few days may be caused, due in part to deficiency of proteins of the right quality rather than to lack in total organic nitrogen

(**10, 30, 31**). Similar conditions may obtain in calcium-deficient plants, except that effects of lack of this element (**30**) are so sudden and drastic that even if carbohydrates are low, the period or extent of proteolytic activity is necessarily very limited.

Sulphur-deficient plants were at all times very high in carbohydrates (page 575); yet the stems of these plants (fig. 1) increased in length, although not in diameter, fully as rapidly as the plants which received the complete nutrient solution. Analyses (tables II and III), however, show that as compared with the complete-nutrient plants, the minus-sulphur group contained on July 8 more than twice the concentration of soluble organic nitrogen and were especially high in amino acids, amide, and ammonia. Further, the organic sulphur in the complete-nutrient plants was nearly all in a complex protein form; whereas, in the minus-sulphur series, it was mainly water-soluble, protein-free organic sulphur, only a very small part of which was cysteine or glutathione.

The initial tomato plants, which were strongly vegetative and actively growing, contained much soluble organic sulphur and gave a strong S-H reaction (page 570). This is in striking contrast to the quality of the soluble organic sulphur of the minus-sulphur plants, which included practically no S-H sulphur. The low concentration of soluble organic sulphur in the only moderately vegetative plus-sulphur plants is not entirely without precedent.[5] AITKEN (**1**) analyzed fresh blade tissue of five different grasses and states that the water-soluble "protein" fraction did not contain sulphur. It should be mentioned, though, that his method of harvesting for analysis resulted in an analytical sample of grass blades which apparently did not include active basal growing tissues. Likewise in apple (**22, 36**), in *Fagus* (**7**), and in *Salix* (**36**), limited data give some suggestion that sulphur in these plants may be in large part insoluble, especially in mature leaves and storage tissue.

As usual, the plants which received sulphate but no nitrogen in the nutrient solution (minus-nitrogen plus-sulphur series) were very high in carbohydrates, and were making no measurable growth when exposed to seasonal light conditions of the greenhouse; but during the period of darkness accompanying decrease of carbohydrates, the stem increased in length by 15 to 20 cm., protein decreased, and the concentration of soluble organic nitrogen approximately doubled (tables VIII and IX). The changes in sulphur fractions are less, but indicate definitely a marked de-

[5] Drying plant tissue may result in a marked increase in soluble organic sulphur and a corresponding decrease in protein sulphur. Various workers have used dried plant tissue in making analyses for these fractions, but the results would appear impossible of interpretation so far as protein and soluble organic sulphur are concerned.

crease in sulphur-containing proteins. (Stems only were analyzed; soluble organic sulphur formed proteolytically was presumably translocated to other organs.)

In contrast it would seem that the sulphur-deficient tomato plants were very actively breaking down sulphur-containing proteins, even under conditions of extreme carbohydrate accumulation. The effects of treatment in darkness on the minus-sulphur plants are in complete harmony with these results. When minus-sulphur plants were placed in darkness, there was a marked drop in carbohydrates but no perceptible change in rate of stem elongation as compared with minus-sulphur plants continually in the light, and no material change in concentration or quality of sulphur fractions (tables VIII and IX). Expressed on a green-weight basis, it is seen that changes in nitrogenous fractions were also slight, although, owing to loss in dry matter, the percentage of nitrogen as well as of sulphur shows an increase.

Proteolytic activity in plants (**8, 26, 29**) seems generally to be intimately associated with decrease in storage carbohydrates. Obviously, however, proteolysis in the sulphur-deficient tomato plants was not limited by the presence of a high concentration of carbohydrates. An explanation is not apparent, but it was this capacity on the part of the sulphur-deficient tomato plants which made possible extensive reutilization of organic sulphur.

Whether or not sulphates may be formed proteolytically is uncertain, although there is some evidence to indicate that this may occur (page 591).

COMPOSITION IN RELATION TO ANATOMY.—Tomato plants have previously been grown in a deficiency of nitrogen (**20, 29**), phosphorus (**10**), potassium (**30**), and calcium (**31**). At various stages of growth, and under different conditions of environment and nutrition, the concentration of carbohydrates has been found to vary widely. Accumulation of carbohydrates, whatever the cause, has been found to be associated with thick cell walls in xylem, phloem region, and cortex; whereas a low concentration of carbohydrates has been found to be associated with comparatively thin walls in these tissues. Similar conditions have been reported by WELTON (**47**) for oats and wheat. Tomato plants grown in a deficiency of sulphur are no exception to this generalization. The minus-sulphur plants of the present experiments were very high in carbohydrates; even the pith cells of the stem tip were thick-walled, although not so thick as the conductive elements of the xylem, fibers of the phloem region, and collenchyma of the cortex. It would seem that the thickness of cell walls is intimately associated with the supply of available carbohydrates, and only indirectly (with

the possible exception of calcium[6]) with the supply of various essential elements obtained from the soil.

On the other hand, the characteristics of the protoplast appear to be rather definitely modified by nutrient treatment. Plants low in nitrogen, but with opportunity for abundant photosynthetic activity, have little meristematic tissue but are not injured. A tomato plant may, under advantageous conditions, remain alive for at least two years without external application of nitrogen. The protoplasm, where present, gradually becomes less dense but is highly refractive and does not look opaque. Proteinaceous materials of the protoplasm may be extensively reutilized in the formation of new meristematic tissue; but this apparently occurs, as already mentioned, only when accompanied by a decrease in carbohydrate reserve.

This description applies equally well to minus-sulphur tomato plants, with the single exception that in sulphur deficiency, even though carbohydrates are high, sulphur-containing nitrogenous constituents of the protoplast are constantly being reutilized for the development of progressively smaller stem and root tips. In either nitrogen or sulphur deficiency, proteinaceous protoplasmic material is limited in amount, but there does not appear to be radical disintegration of the protoplast, as is found in phosphorus (**10**) and calcium (**31**) deficiency, nor deposition of the abnormal granular proteinaceous particles frequently observed in plants lacking potassium (**30**), phosphorus (**10**), or calcium (**31**).

It would seem from the results of these experiments with tomato, and from the work of others (**16, 17, 18, 46, 48**), that cysteine and glutathione or similar compounds are essential constituents of actively dividing cells. At least, meristematic tissues were not observed that did not contain higher concentrations of S-H sulphur than adjacent mature cells. It should be emphasized, however, that many other materials are equally essential. In fact, as already mentioned, sulphur deficiency in tomato was less drastic in its effect than limitation of other essential elements.

NITRATE ASSIMILATION AND CARBOHYDRATE ACCUMULATION.—Some tomato plants were grown with no external supply of either sulphate or nitrate. They were shifted to a solution containing nitrate but no sulphate, and the plants absorbed nitrate instantly. This experiment demonstrates that for nitrate absorption the presence of sulphate in a solution is unnecessary. The plants lacking sulphate and nitrate in their nutrient solution soon became

[6] Calcium deficiency in algae has been said (35) to prevent the formation of the middle lamella. In tomato (31), however, lack of calcium did not prevent the early formation of the middle lamella for which calcium apparently may not be directly necessary. Calcium pectate at later stages may, however, be essential. Especially in the fibrous roots of tomato, there was found a secondary effect of calcium deficiency that resulted in dissolution of the middle lamella and separation of cells.

exhausted of the nitrate initially present in the plant. Sulphate, however, was retained as such, and, following complete assimilation of nitrate, did not materially decrease in concentration. As might be anticipated, it was found practically impossible to obtain plants simultaneously deficient in sulphate and nitrate. However, absorption of nitrates does not appear to have been a limiting factor in the minus-sulphur tomato plants of these experiments, as they were consistently much higher in nitrate than the plants which received the complete nutrient solution (tables II and III).

Nitrates accumulated in the sulphur-deficient plants because the assimilation or synthesis of nitrates to amino acids and other organic nitrogenous compounds was greatly inhibited. ECKERSON (**13**) found that the tomato plants lacking sulphur were low in reducase (nitrate reducing material); the reducase was maintained at low level, allowing continuous but slow reduction of nitrate. Also in a few hours following the shift from minus- to plus-sulphur nutrient solution, the plants gave strong reactions for nitrites; and eleven days after receiving sulphate there was a marked decrease in carbohydrates. Carbohydrate accumulation is not unfavorable to nitrate reduction; in fact, typical low-nitrogen plants are very high in carbohydrates, and especially high in reducase activity (**9**). Accumulation of carbohydrates was not caused by the fact that sugars could not be translocated, nor accumulation of starch by the fact that digestion to sugars was seriously limited. When the plants were placed in darkness, starch was freely hydrolyzed to sugars. Starch and sugars were also present in very large quantities in all parts of the plant, even in stem and root tips. Obviously, translocation of carbohydrates could not have been more complete.

One of the principal uses of carbohydrates, however, is in protein synthesis. Carbohydrates and nitrates (tables II and III) undoubtedly accumulated in the sulphur-deficient plants because there was little synthesis of amino acids or other proteinaceous materials. In this respect, the low-sulphur plants are similar to tomato plants lacking phosphates (**10**) or potassium (**30**), although the response differs in degree (**13**).

NITRATE AND SULPHATE ASSIMILATION.—The assimilation of nitrates in plants (**9**) involves reduction to nitrates and ammonia, following which there is oxidation of sugars and synthesis of amino acids. In the tomato this process appears to be carried on in the more alkaline phloem region of roots and tops. In certain other plants, such as apple (**11**), narcissus (**28**), and asparagus (**26**), the reduction of nitrates is restricted mainly to the fibrous roots; and under favorable conditions of growth, nitrates are seldom found in the tops of these plants because they are synthesized to amino acids and other organic nitrogenous compounds in the roots.

An attempt was made to determine whether sulphate and nitrate assimilation were in any respect similar. ECKERSON found that the minus-sulphur

tomato plants were very high in material that reduced sulphates to sulphites. Likewise, when the minus-sulphur plants were shifted to plus-sulphur treatment, sulphites appeared in considerable quantity, especially in the phloem of the tops and to a less extent in similar tissue of the roots. Shortly following the appearance of sulphites, unusually strong reactions were obtained for S-H sulphur, including cysteine. Glutathione also was probably present. The S-H sulphur reactions were largely restricted, however, to the phloem and cambium and to the meristematic tips of stem and roots, a condition that has frequently been observed by others (**16, 17, 19, 48**). No cystine was observed. Undoubtedly there were present other unidentified compounds of soluble organic sulphur such as possibly methionine, a comparatively recently discovered sulphur-containing amino acid (**3, 25**). There was certainly much soluble organic sulphur in the minus-sulphur plants (tables II and III), even more than before shifting to plus-sulphur treatment; but practically none of it was in the form of cysteine, glutathione, or cystine.

In tomato, therefore, sulphate and nitrate reduction seem in certain respects to be similar, in that plants deficient in sulphur are most active in sulphate reduction, and plants deficient in nitrogen are especially active in nitrate reduction. The region of reduction in both cases is the phloem, and may take place in either roots or tops.

It has been pointed out that in asparagus nitrate reduction takes place in the roots and that nitrates are not usually found in the succulent actively growing stems or spears. Some spears were analyzed and found to contain, on a green-weight basis, 0.036 per cent. sulphate sulphur and traces of sulphite. The spears contained no nitrates. Some potted asparagus plants were placed in a chamber in darkness at 10° C. As usual (**27**) this temperature prevented assimilation of nitrates and they appeared in the spears, but there was no increase in concentration of sulphates. A field-grown apple tree was examined and found to contain nitrates in the fibrous roots only, yet sulphates and sulphites were found in roots, twigs, petioles, and leaves. Likewise, ECKERSON found that in sulphate reduction the tops of apple trees were even more active than the roots, although the reverse was true for nitrate reduction. A sample consisting of twigs and petioles was found to contain, on a green-weight basis, 0.018 per cent. sulphate sulphur, but no nitrates. It is apparent from tables X and XI that narcissus contains nitrate in the roots only, whereas sulphate is present in all parts of the plant.

In these plants, therefore, sulphate assimilation does not appear to be restricted to the roots. Most of the assimilation of nitrates, however, takes place in the roots.

GROWTH OF TOMATO AS ASSOCIATED WITH CONCENTRATION OF NITRATE AND SULPHATE.—Tables IV and V show that the leaves and fruits of the minus-sulphur plants were comparatively low in sulphate-free sulphur. This is not true of the stems and roots, however, especially on a percentage of green-weight basis. Likewise, the data of tables II and III show that the whole stems of minus-sulphur plants were, at all times during the experiment, practically as high in concentration of total organic sulphur as the plants which received and contained an abundance of sulphate.

The sulphur-deficient plants certainly were not low in total soluble organic sulphur (tables II and III) obtained apparently exclusively through the breaking down of proteins (page 583). However, in spite of the high concentration of total soluble organic sulphur, the plants contained practically no cysteine or glutathione. If these or other essential sulphur-containing materials are formed chiefly through sulphate reduction and not proteolytically, it would seem to explain the apparent need for maintaining in the tomato plant, during its period of active growth, a somewhat high concentration of sulphate.

Repeated experiments (**20, 29, 30**) involving nitrogen nutrition with nitrates as the sole source of nitrogen show that, for vigorous growth of tomato, there must be maintained in the plant a high concentration of nitrates. The concentration must be far in excess of that which will be assimilated or the plant does not grow vigorously. Nitrates in themselves, however, are not essential for growth; a vigorous nitrate-free tomato plant may easily be obtained by maintaining a lower percentage of ammonium (**42**). It may be that in nitrate or sulphate nutrition of tomato, a considerable quantity of the unelaborated ion must be present to furnish the plant at sufficient rate an adequate amount of certain intermediate products of protein synthesis. In this connection it may be pointed out that HAMMETT (**17**) has shown that low concentrations of S-H compounds in a nutrient solution greatly accelerated growth of roots of seedlings of *Zea mays* and *Phaseolus vulgaris*. It appears probable that in plant nutrition S-H sulphur bears a relationship to sulphate in some respects similar to that of ammonium to nitrate.

There appears to be no information available as to the possible physiological value of an abundance of sulphate[7] and nitrate in tomato. It is certain, however, that lack of sulphate and nitrate cannot be a limiting factor in the synthesis of organic compounds for which nitrogen and sulphur are necessary, so long as sulphate and nitrate are present in abun-

[7] Sulphates do not appear to be held by proteins or other ampholytes of tomato as tenaciously as some other ions, as for example calcium (**31**), nitrate (**42**), or ammonium ions (**42**). Electrodialysis (**23**) of tomato stem tissue failed to remove more sulphate than the usual method (**5**) of aqueous extraction at approximately pH 5.6.

dance. The initial plants, which were high in sulphate and nitrate (tables II and III), gave exceptionally strong S-H reactions, contained some cystine (page 570), and for complete-nutrient plants were comparatively high in sulphate-free soluble sulphur. There are, however, analyses (**18**, **32**, **41**) tending to show that the sulphate content of plants may be low, especially when the plants are nearly mature (**41**). Whether or not a strongly vegetative tomato plant might be obtained that was low in sulphate seems doubtful. It would have been worth while to have determined effects of giving tomato plants a nutrient solution containing no sulphate but instead a low concentration of S-H sulphur continually supplied. Chemicals in sufficient quantity for such nutrient treatment were not available, however. At least it is clear that the tomato plants of these experiments decreased greatly in vegetative activity before there was extreme depletion of sulphate (fig. 1, tables II and III).

On the other hand, high-protein narcissus bulbs (**28**) containing no nitrates, and with no external source of nitrogen, may grow vigorously for months with a nitrogen supply obtained solely through the utilization of storage proteins. The dormant narcissus bulbs which were studied contained, however, considerable quantities of sulphate (tables X and XI).

It was thought that seeds might be obtained which were free of sulphate and that with them a test might be made to see whether cysteine or other S-H compounds were formed proteolytically. Soy beans were employed, and, although the dormant seeds contained insufficient sulphate for a macro-determination (**2**), the benzidene reaction indicated traces of sulphate. There appeared to be practically no S-H sulphur in the dry seeds. Three hours after integuments had been broken and the seeds had been moistened with distilled water, there were noticeable quantities of sulphate and sulphite (benzidine and tetrathionate reactions respectively, page 568). Fairly strong reactions for S-H compounds were also obtained at that time, particularly in the region of the hypocotyl and epicotyl. The germinating seeds were watched at frequent intervals for a period of five days, but there was no noticeable change in concentration of sulphite or S-H sulphur. But at the end of the five-day germination trial, the quantity of sulphate sulphur in the seeds was found by macro-analysis (**2**) to be 0.006 per cent. This percentage figure is based upon the original air-dry weight of the seeds.

So far as the observations on reduced sulphur are concerned, these results are in agreement with those of VIVARIO and LECLOUX (**45**). The question as to whether S-H compounds were formed synthetically from sulphites or wholly or in part through proteolysis may not be answered at this time. Nitrates appear never (**9**, **26**, **27**, **28**, **29**) to be formed proteolytically, yet phosphates and probably ammonium may be formed from the breaking down of phosphatides (**10**). The presence of sulphatides (**21**)

has not been demonstrated in plants. However, catabolic changes in organic sulphur-containing compounds would appear to be a possible explanation of the appearance of sulphates in the germinating seeds of soy beans.

In this connection attention may be called to the fact that traces of sulphate (tables II and III) were present in the minus-sulphur tomato plants. Even as late as July 8, an occasional crystal of benzidene sulphate formed on addition of the benzidene reagent to sections of the stem tips of the sulphur-deficient plants. There was, however, no sign of disorganization of the protoplast. In low-phosphorus tomatoes, breaking down of phosphatides to phosphates is accompanied by definite injury to the cells, and death soon follows (**10**).

Summary

1. Symptoms of sulphur deficiency in tomato developed slowly in plants which lacked an external sulphate supply. The plants looked as though they had been gradually but not completely deprived of nitrogen. The lower leaves were yellowish green, the stems were hard and woody, and the roots were extensive. Both roots and stems were of very small diameter.

2. These characteristics may also be exhibited by tomato plants deficient respectively in nitrogen, phosphorus, or potassium. The sulphur-deficient tomato plants had, however, a remarkable capacity for stem elongation; and although the stems were woody and thin they increased in length, but not in diameter, as rapidly as the stems of the complete-nutrient plants.

3. The sulphur-deficient tomato plants were extremely high in carbohydrates, and contained much more nitrate than the plants which received the complete nutrient solution.

4. Carbohydrates and nitrate accumulated in the minus-sulphate treated tomato plants because reduction of nitrates and oxidation of sugars was comparatively slow although not entirely inhibited.

5. Digestion of starch and translocation of sugars took place freely in minus-sulphate tomato plants.

6. Cell wall thickness in tomato plants seems to be more intimately associated with carbohydrate content than with any other single factor of nutrition. The high-carbohydrate, minus-sulphate tomato plants had very thick cell walls and a relatively high proportion of fibers and lignified tissue.

7. Protoplasm of the sulphur-deficient tomato looked much like that of plants lacking nitrogen; that is, the protoplasm was limited in amount but not noticeably injured as in deficiency of phosphorus, calcium, or potassium.

8. Cysteine specifically, probably glutathione, and possibly other S-H compounds were present in meristematic tissue and were especially high

when complete-nutrient tomato plants contained an abundance of sulphate and nitrate.

9. Associated with low content of S-H sulphur in the minus-sulphate tomato plants there was practically no active cambium. Roots and stems were thus of small diameter, but they increased in length as a result of cell division in the apical meristems of stems and roots. The meristematic tissues contained faint traces of S-H compounds.

10. Proteolysis is usually accompanied by decrease in reserve carbohydrates. The minus-sulphate tomato plants were at all times extremely high in carbohydrates, yet complex proteins were rapidly broken down to soluble organic compounds of sulphur, to amino acids, to asparagine, and to ammonia. This proteolytic activity resulted, however, in little if any S-H sulphur.

11. Although the tomato plants lacking an external sulphate supply were not very low in percentage of total organic sulphur, much of it was water-soluble; whereas the organic sulphur of the complete-nutrient tomato plants was mainly in a complex protein form. The soluble organic sulphur in the minus-sulphur plants contained practically no cysteine, glutathione, or cystine. The form of sulphur was not determined.

12. Limited data give some evidence to indicate that sulphate and ammonium may be formed proteolytically. It is known that phosphatides break down in part to phosphates and probably ammonium.

13. In tomato, sulphate is reduced to sulphite and apparently to S-H sulphur in the comparatively alkaline phloem region of roots and tops. This is also true of the reduction of nitrates to nitrites and ammonium.

14. In apple, narcissus, and asparagus, the region of nitrate reduction is largely confined to the fibrous roots. Sulphate reduction in these plants takes place in the roots to some extent, but mainly in the tops.

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